



Immunotoxin Therapy for Mesothelioma

Malignant Mesothelioma

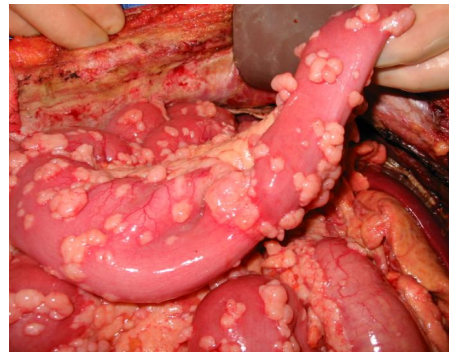


- Tumor of pleura or peritoneum
- About 3,000 new cases in US each year
- Limited treatment options and poor prognosis

Mesothelioma arises at sites that are lined by mesothelial cells



Pleural



Peritoneal



Pericardial



Tunica vaginalis

Asbestos is the primary cause of mesothelioma

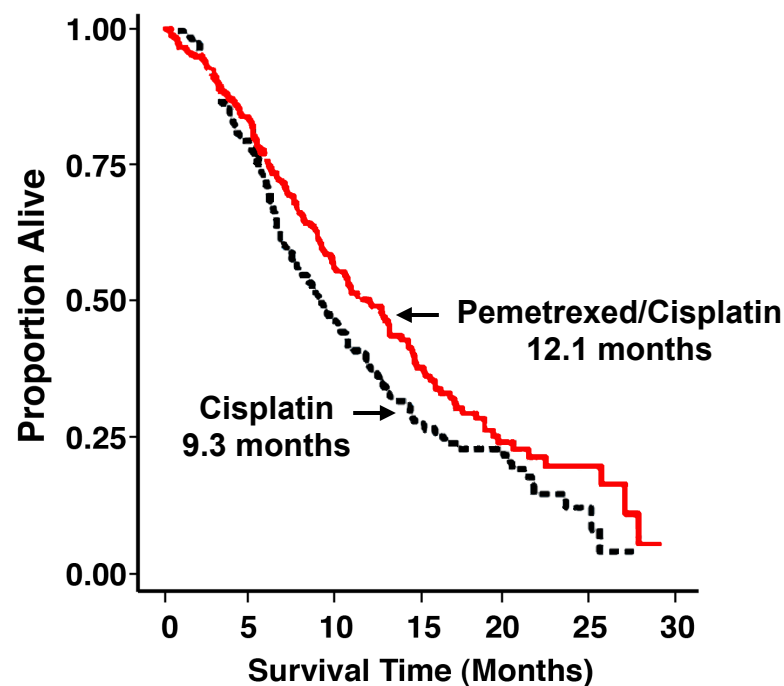
- **In 1960, Wagner reported increased incidence of mesothelioma in asbestos miners of South Africa**
- **Increased incidence in miners, family members of miners, asbestos insulators, defense personnel working in ships**
- **Long latency from the time of asbestos exposure to onset of disease**
- **No history of asbestos exposure in 30-50% of cases of mesothelioma**

Other causes of mesothelioma

- Simian virus 40 (SV40) has been implicated in the etiology of some mesotheliomas
- Increased risk in patients with prior radiation for Hodgkins disease or Non-Hodgkins Lymphoma
- **BRCA1 associated protein-1 (BAP1)**
 - Germline *BAP1* mutations in two families with high incidence of mesothelioma
 - Germline BAP1 mutations in 2/26 sporadic mesotheliomas
 - Both individuals had prior uveal melanoma

Pleural mesothelioma is an aggressive disease with poor prognosis

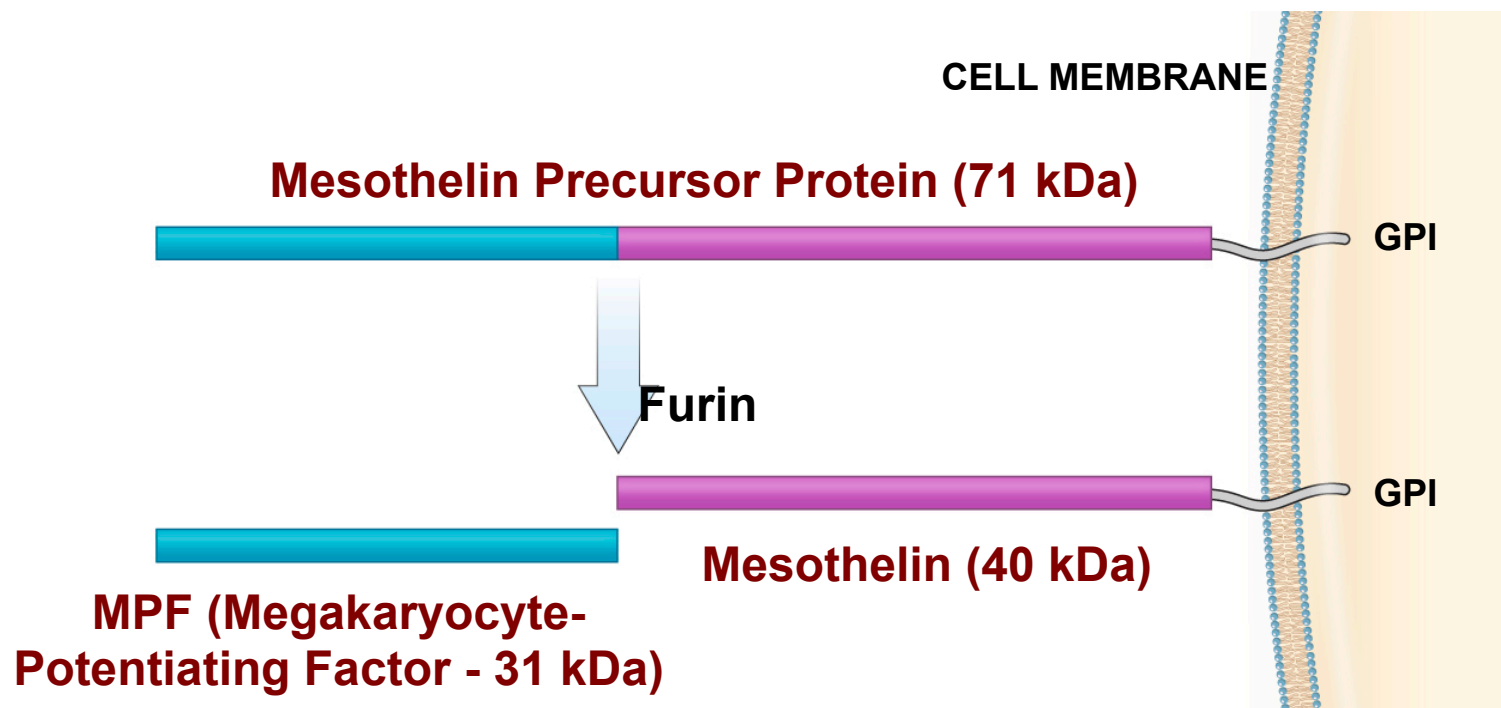
- Surgery as part of multi-modality therapy prolongs survival in selected patients
- Most chemotherapy drugs are ineffective
- Pemetrexed plus cisplatin is the only FDA approved treatment



Vogelzang et al., JCO 2003

Mesothelin

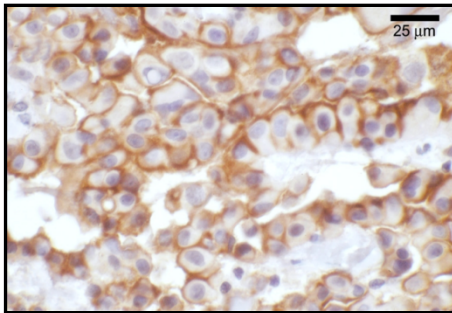
- Is a cell surface glycoprotein
- Expression in normal human tissues limited to the mesothelial cells lining pleura, peritoneum and pericardium



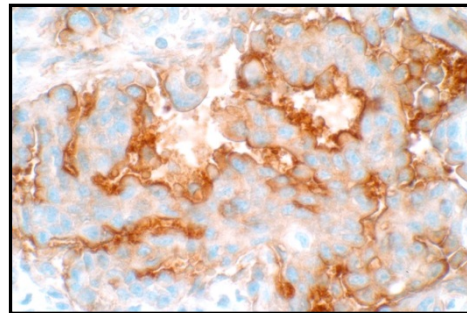
Chang K, Pastan I., PNAS 1996
Yamaguchi N, J Biol Chem, 1994
Pastan, Hassan., Cancer Res. 2014

Mesothelin expression in human tumors

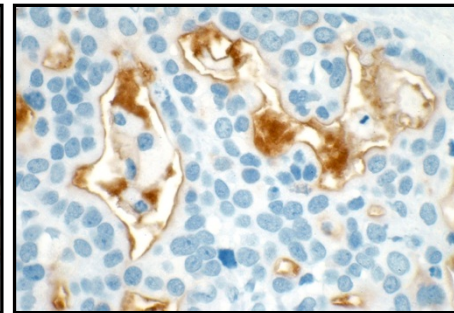
- **Mesothelioma** ~ 100%
- **Pancreatic Cancer** ~ 100%
- **Ovarian Cancer** 67-71%
- **Lung adenocarcinoma** 41-53%
- **Gastric cancer, synovial sarcomas, biliary cancers**



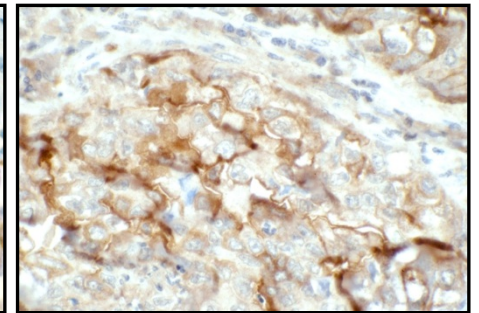
Mesothelioma



Ovarian Cancer



Pancreatic Cancer



Lung Cancer

Hassan et al. Clin. Cancer Res., 2004
Ordonez NG. Am J Surg Pathol, 2003

Mesothelin function

- **Biological function of mesothelin is not known**
- **Mutant mice lacking mesothelin gene showed no phenotype and reproduced normally**
- **Mesothelin is a novel CA125 (MUC16) binding protein and may play a role in tumor implantation in the pleural or peritoneal cavity**
- **MPF and mesothelin stimulate the growth of the lung cancer cell line A549 in the peritoneal cavity of mice**

Rump et al. JBC, 2004

Gubbels et al. Molecular Cancer, 2006

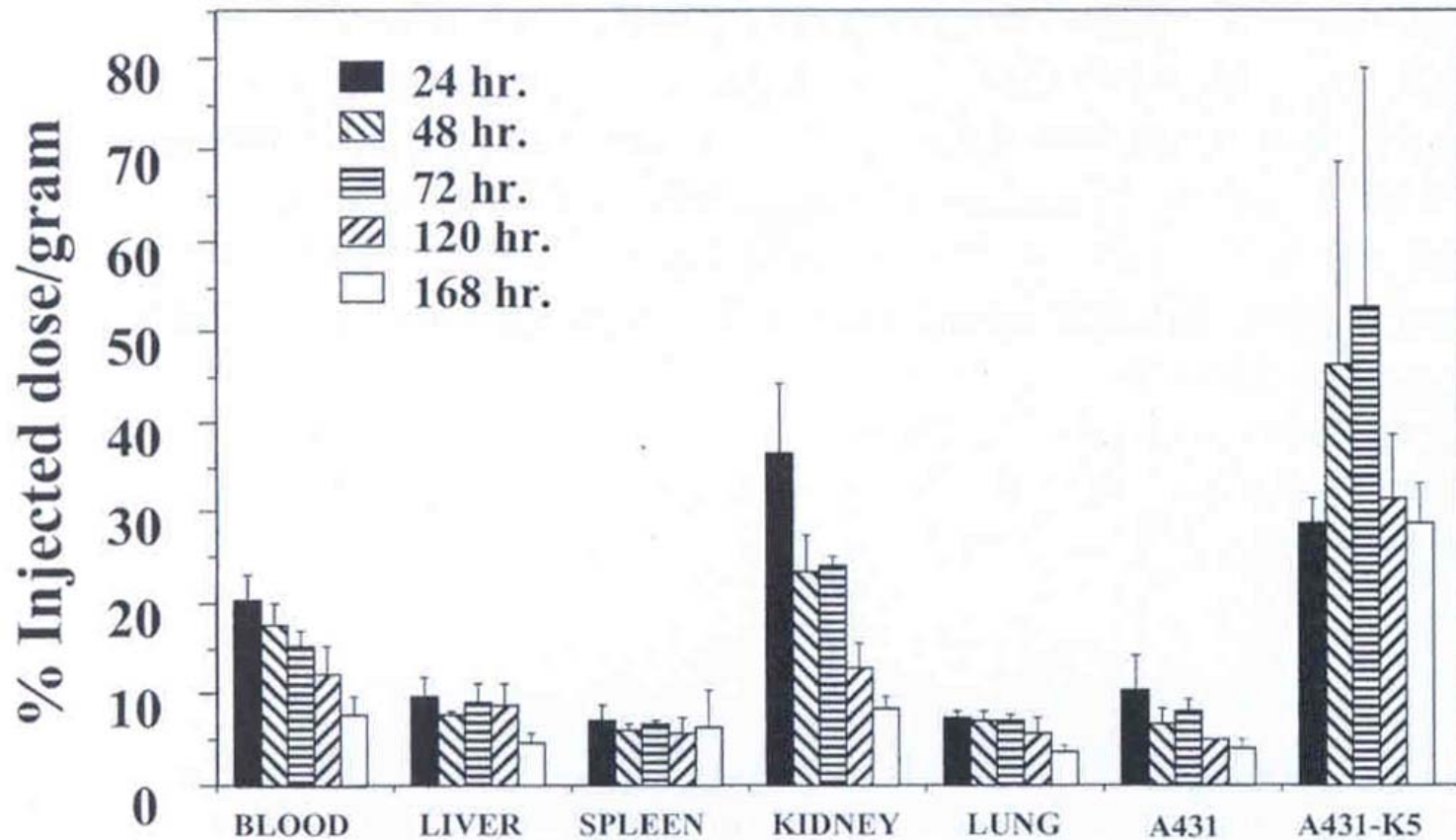
Kaneko et al. JBC, 2009

Zhang et al. Plos One, 2014

Targeting mesothelin for cancer therapy

- High cell surface expression in many solid tumors makes it a good target for antibody based therapies**
- Expression on pleura, pericardium and peritoneum was a concern when we decided to exploit it as a target for cancer therapy**

Anti-mesothelin monoclonal antibody K1 localizes to mesothelin expressing tumors

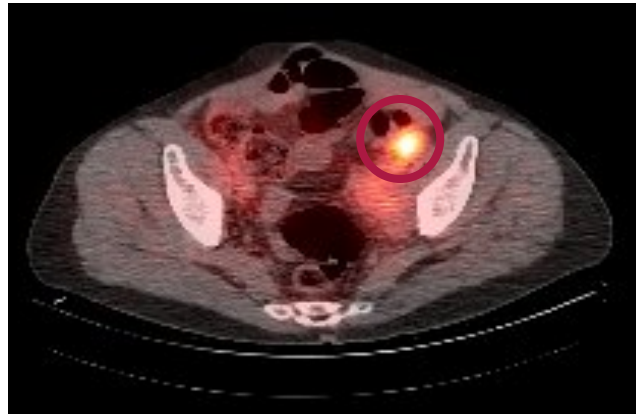


Hassan R et al. Int. J. Cancer, 1999

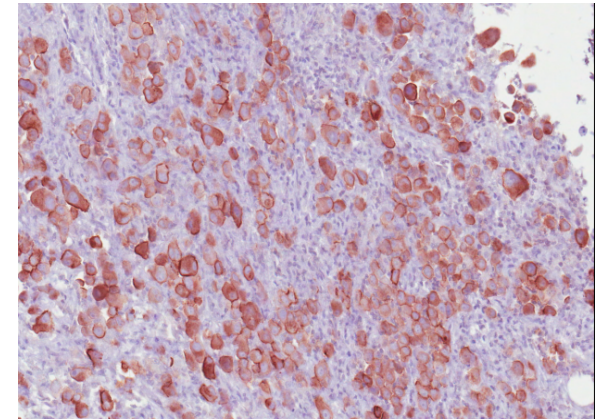
Anti-mesothelin antibody- Amatuximab localize to mesothelin expressing human cancers



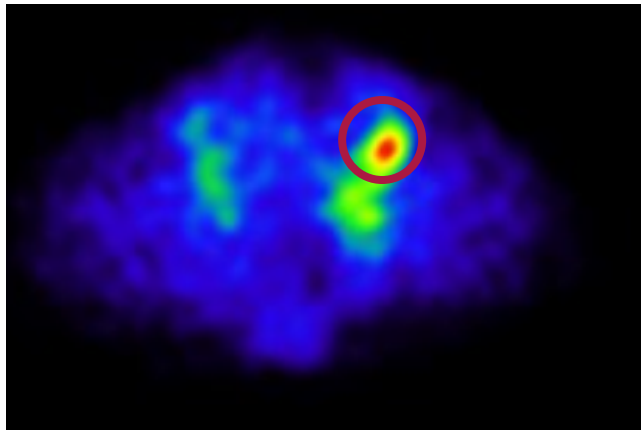
CT scan



PET scan



Mesothelin IHC



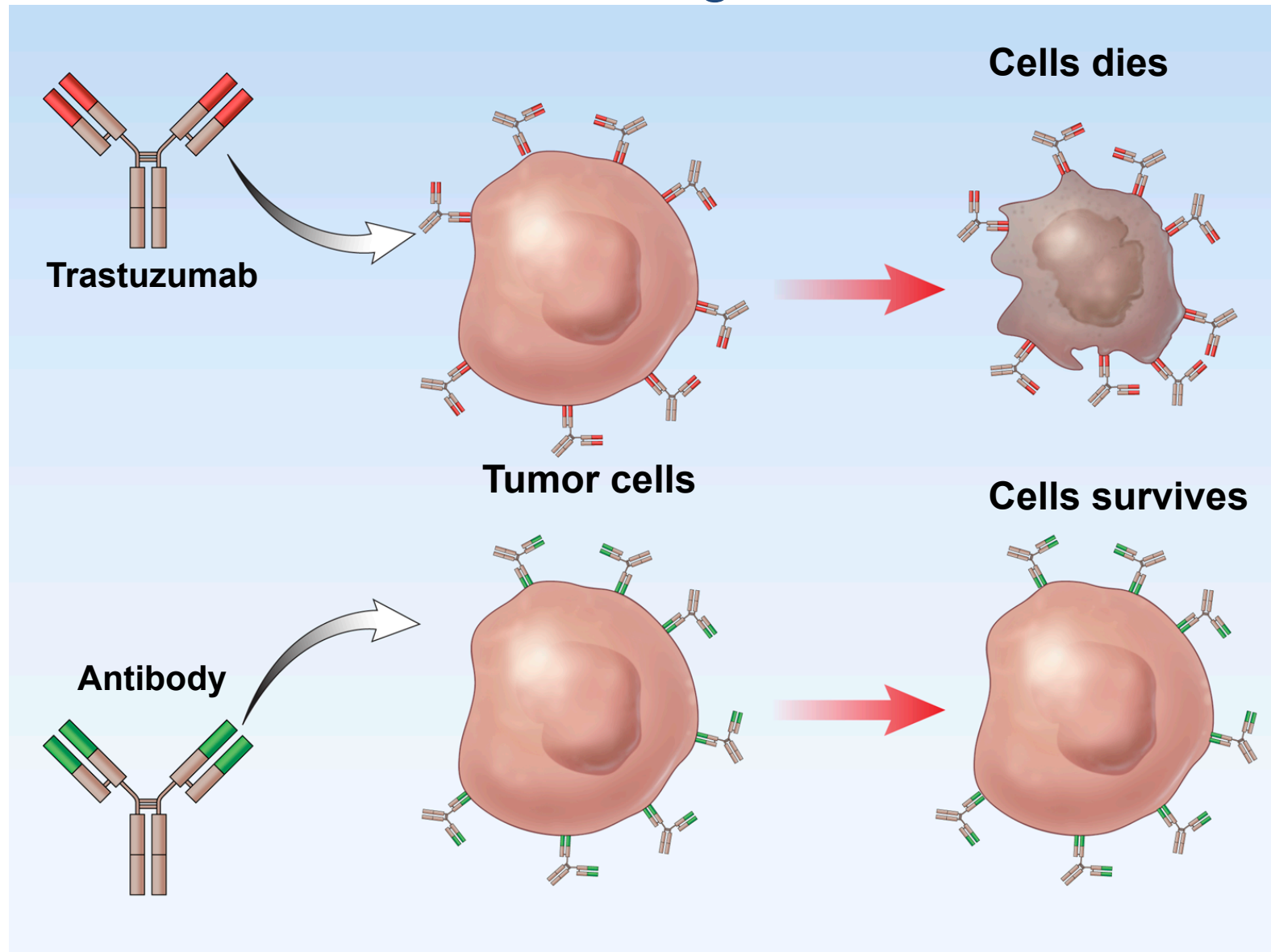
SPECT imaging

Uptake of ^{111}In -MORAb-009 in a patient with metastatic mesothelioma

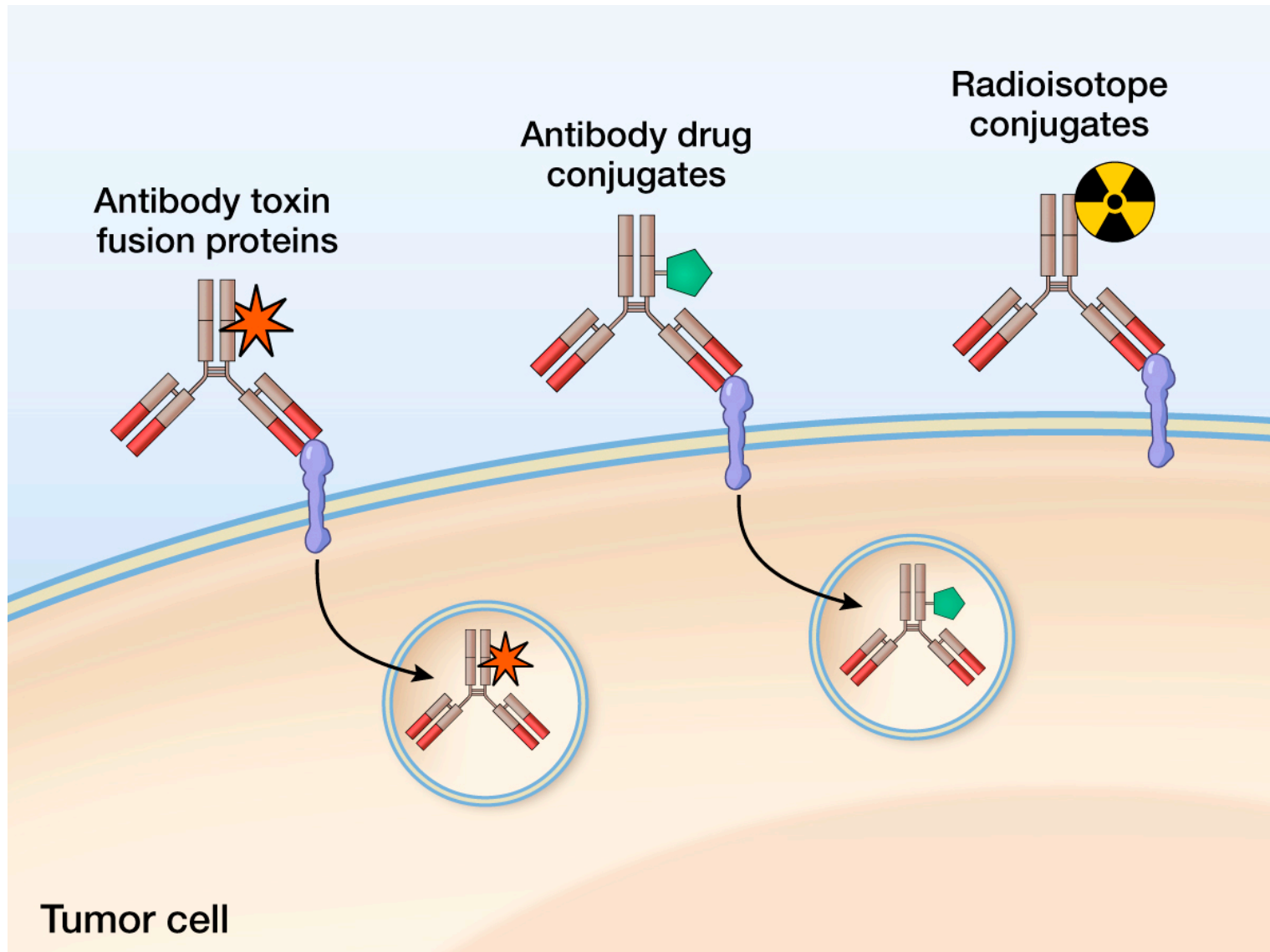
Lindberg,...Hassan., Oncotargets (*in press*)

Targeting mesothelin expressing cancers using an immunotoxin

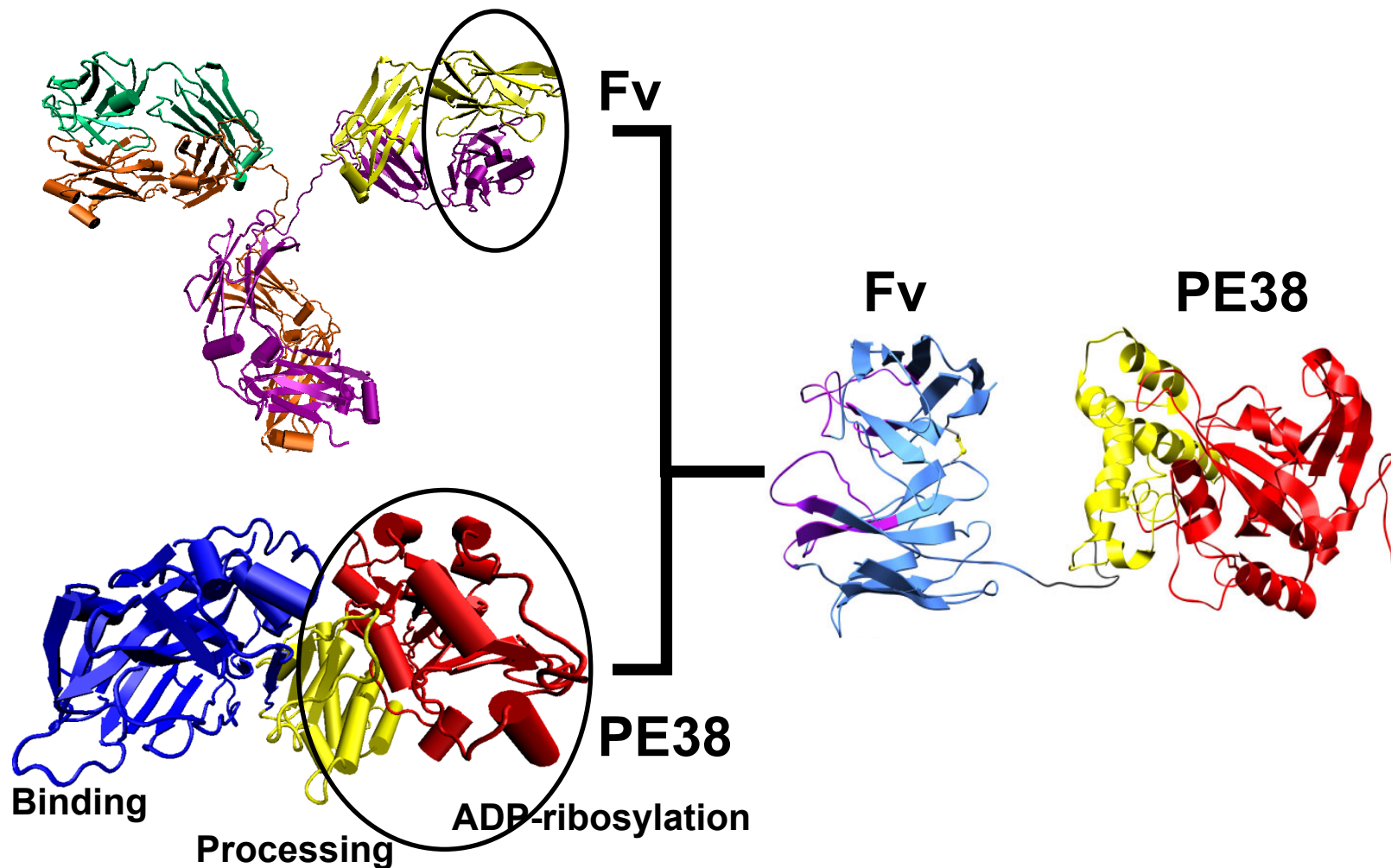
Very few anti-tumor antibodies by themselves are effective in killing cancer cells



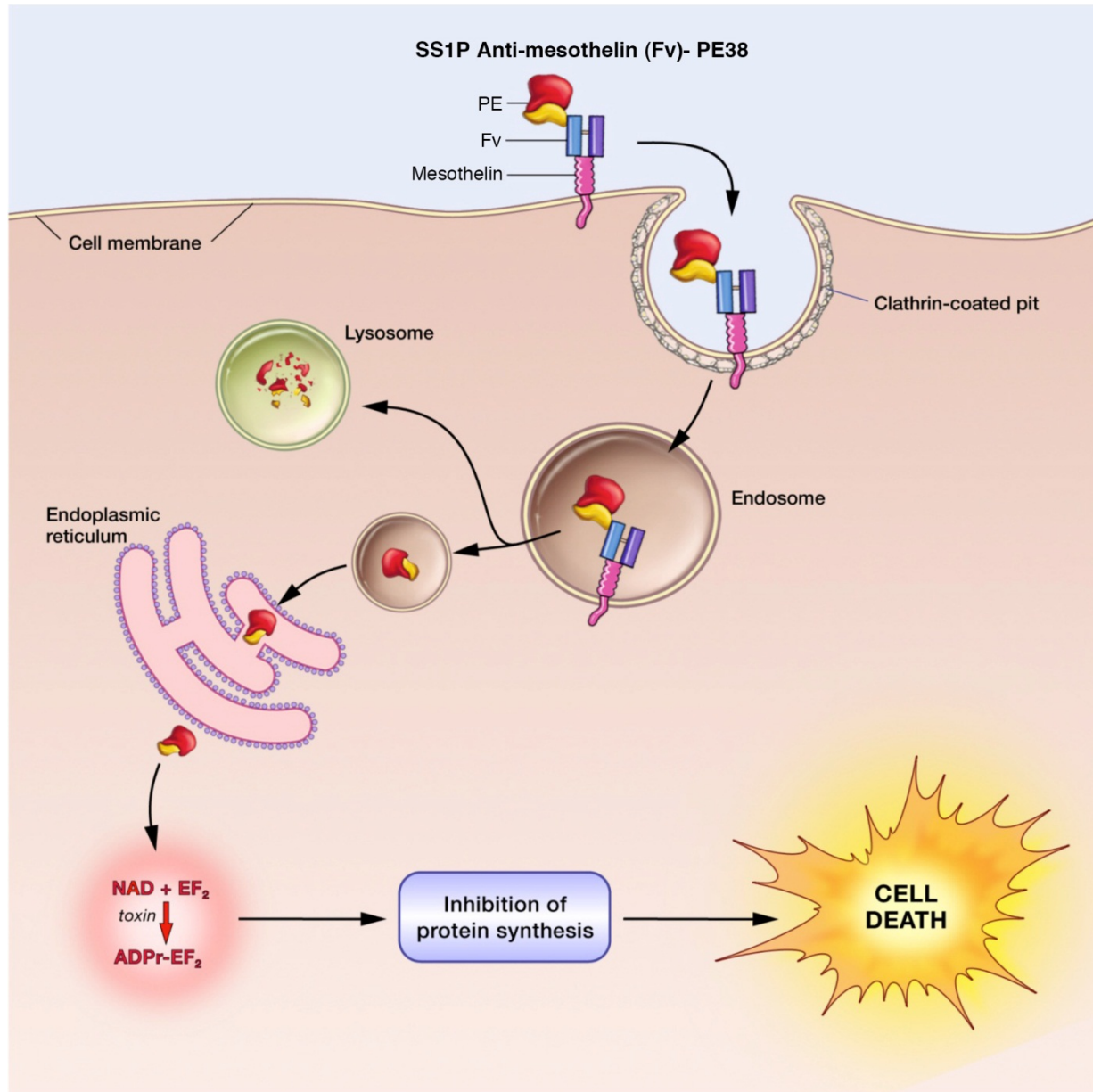
However, mAbs as immunoconjugates can specifically target and kill tumor cells



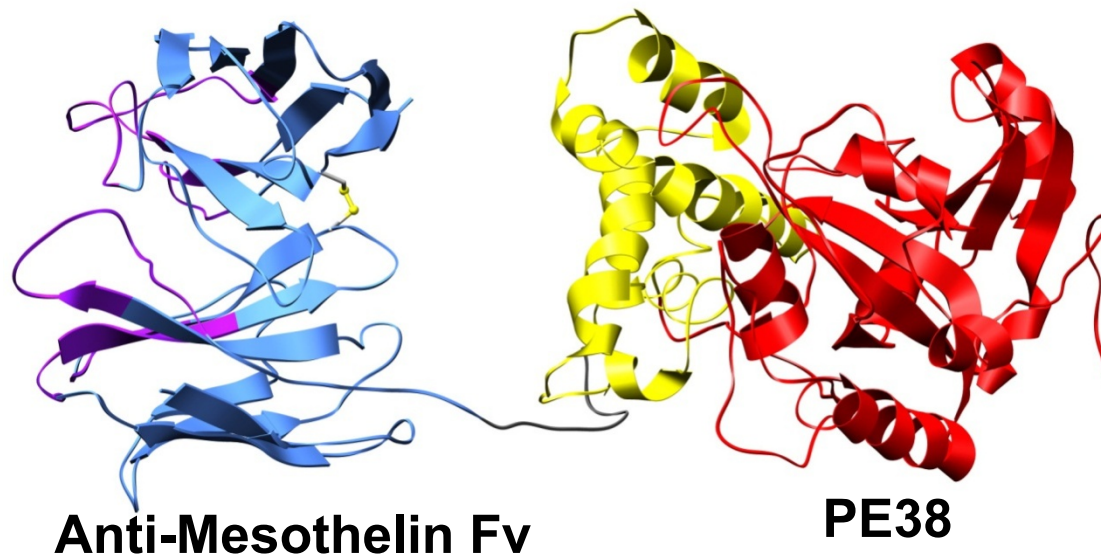
A recombinant immunotoxin consists of an antibody or antibody fragment linked to a toxin



How immunotoxin kills tumor cells



SS1P (Anti-mesothelin Immunotoxin)



- High affinity for mesothelin
- Cytotoxic to mesothelin expressing tumor cells of patients with mesothelioma and ovarian cancer
- Regression of mesothelin positive tumors in mice

SS1P Phase I Trial

- Patients with mesothelin expressing cancers who had failed standard therapies were treated
- MTD was 45 µg/kg; DLT was pleuritis
- Minimal anti-tumor activity with no PR or CR
- SS1P was **immunogenic**
- 30/34 (88%) patients developed neutralizing antibodies after cycle 1

Hassan R et al. Clin. Cancer Res., 2007
Kreitman R et al. Clin. Cancer Res., 2009

The immunogenicity problem of immunotoxins

- **Has limited their development for solid tumors**
- **Previous efforts to overcome this have failed**
- **Felt to be a “huge problem” that cannot be overcome**
- **We have focused on addressing this problem by:**
 - 1. Preventing human immune response to Immunotoxins**
 - 2. Making a less immunogenic immunotoxin**

Preventing human immune response to immunotoxins

Agents that do not work:

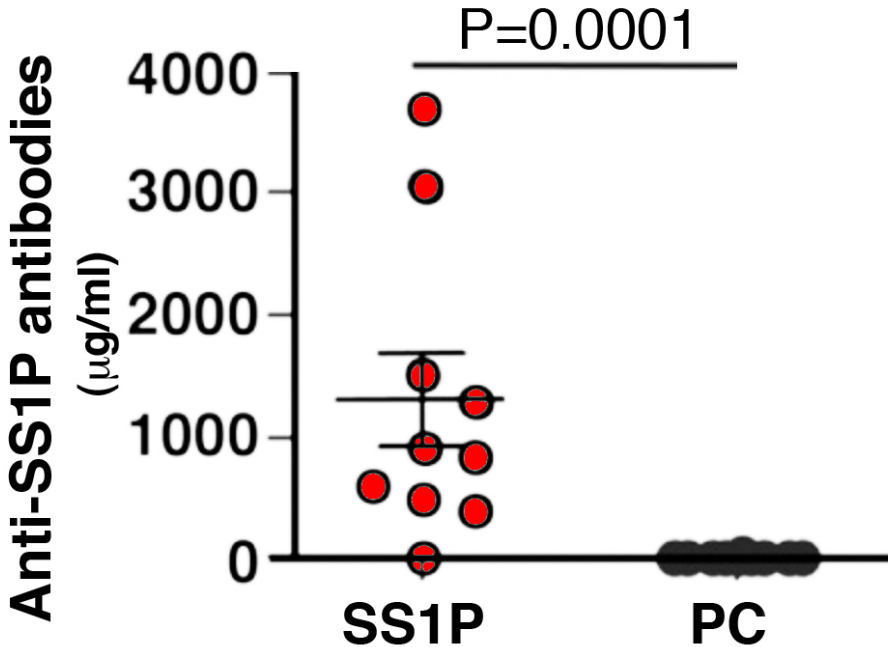
- **Steroids, cyclosporine**
- **Single agent Cytoxan**
- **Rituximab**

**A regimen of pentostatin plus cytoxan to deplete both
T and B cells:**

- **Results in host immune depletion without
myelosuppression**
- **Was safe in trial of patients with renal cancer**

Fowler D., Biol Blood Marrow Transplant, 2011

Pentostatin and cytoxan (PC) prevents the development of antibodies to SS1P in immunocompetent mice



Mossoba et. al. Clin. Cancer Res. 2011

Evaluating SS1P with Pentostatin and Cytosan in patients

- **Rationale:** Depleting host T and B immune cells with pentostatin and cytoxan would decrease formation of anti-SS1P antibodies
- Pentostatin has no efficacy in solid tumors and mesothelioma cells are not sensitive to pentostatin
- Cytosan has no anti-tumor activity in patients with mesothelioma

Pilot study of SS1P with pentostatin and cytoxan in advanced mesothelioma

- **Previously treated patients with treatment refractory and progressive disease**
- **Determine safety and feasibility of this regimen**
- **Primary end-point: To determine if this regimen can reduce anti-SS1P antibody formation**

	Cycle 1 (day 1 to 30)																														Cycle 2 & 3 (day 1 to 21)																				
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Cytosan (200 mg/day)	<div>(days 1-12)</div> <div></div>																														<div>(days 1-4)</div> <div></div>																				
Pentostatin (4 mg/m ²)	1				5				9																						1																				
SS1P (35 or 45 µg/kg)										10		12		14																		2		4		6															

Protocol Schema

Pilot study of SS1P plus PC in advanced mesothelioma:

Patient characteristics

n = 11

Age (yrs.) **54 (43-67)**

Sex **7 M; 4 F**

Pleural meso. **9**

Peritoneal meso. **2**

**Median # of
prior therapies** **3**

Adverse events

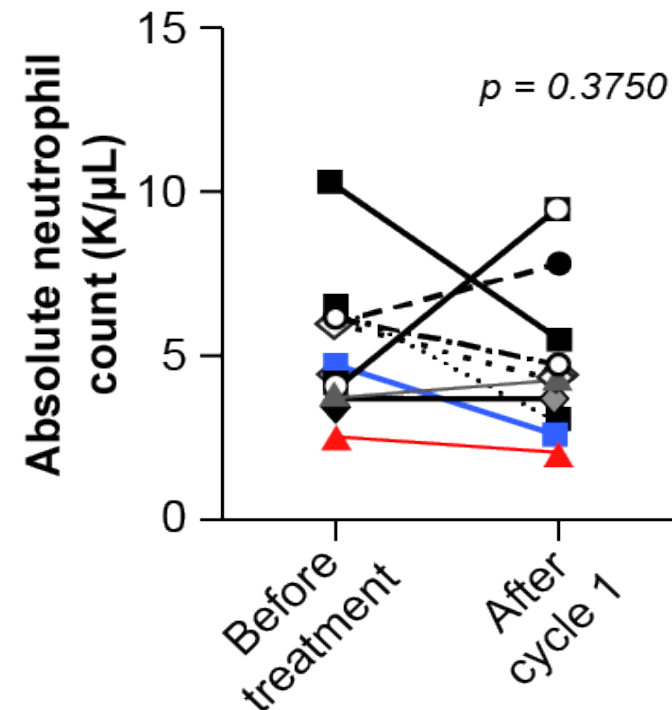
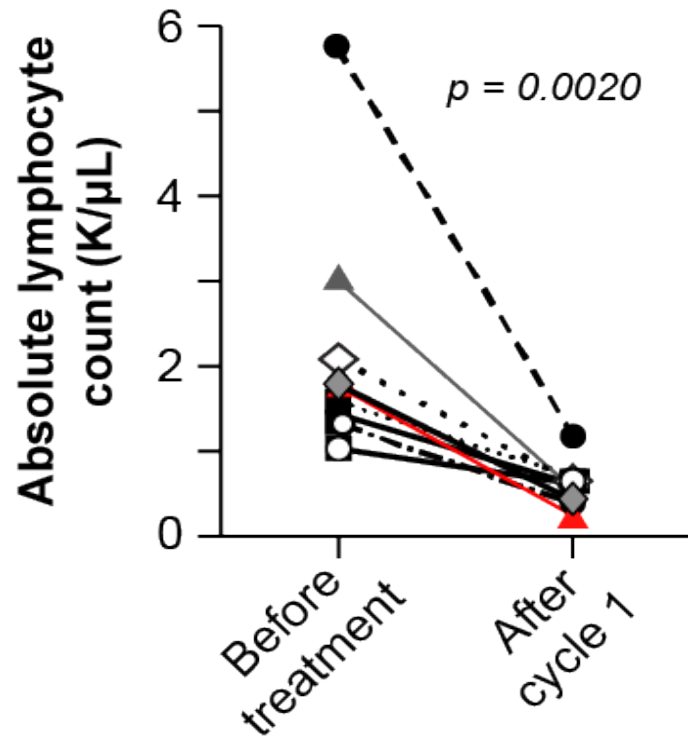
Pentostatin and cytoxan

- **Grade 4 lymphopenia (100%)**
- **Grade 3 anemia (9%)**
- **Grade 3 transaminitis (18%)**
- **Grade 3 fever (9%)**

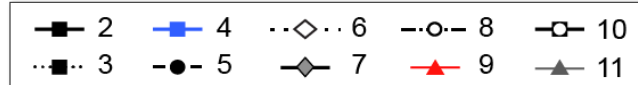
SS1P

- **Grade 3 pleuritic pain, non-cardiac chest pain and back pain (9% each)**
- **Other grade 1, 2 adverse events: fatigue, edema, hypoalbuminemia**

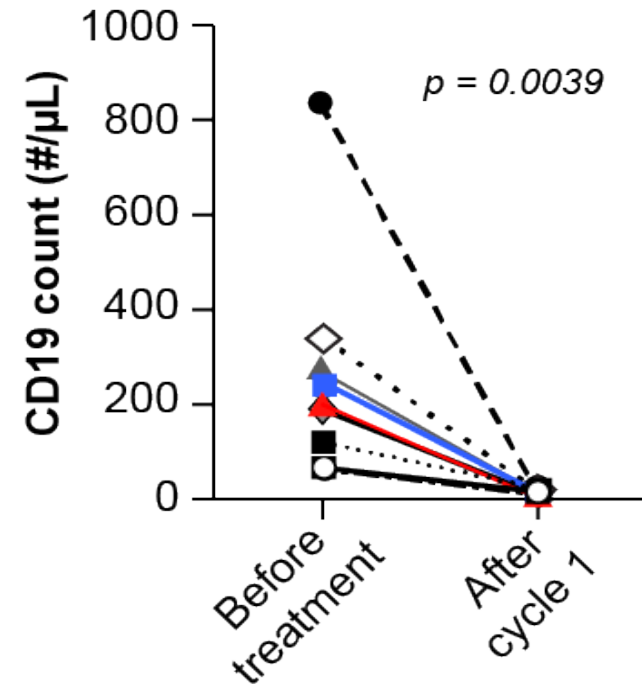
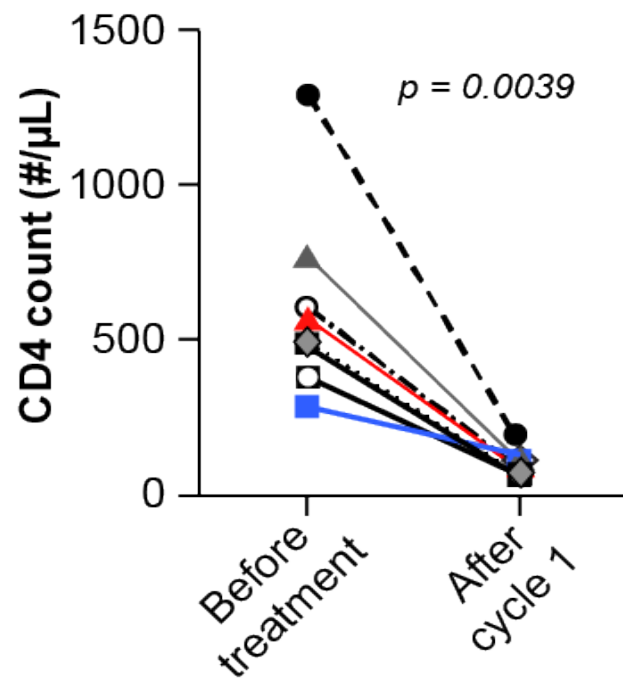
Pentostatin and cytoxan cause lymphodepletion without myelosuppression



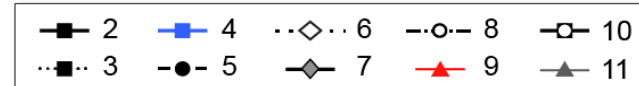
Patients



Pentostatin and cytoxan decrease both T and B Cells



Patients



Pentostatin and cytoxan decrease formation of neutralizing antibodies against SS1P:

Anti-SS1P antibodies after cycle 1

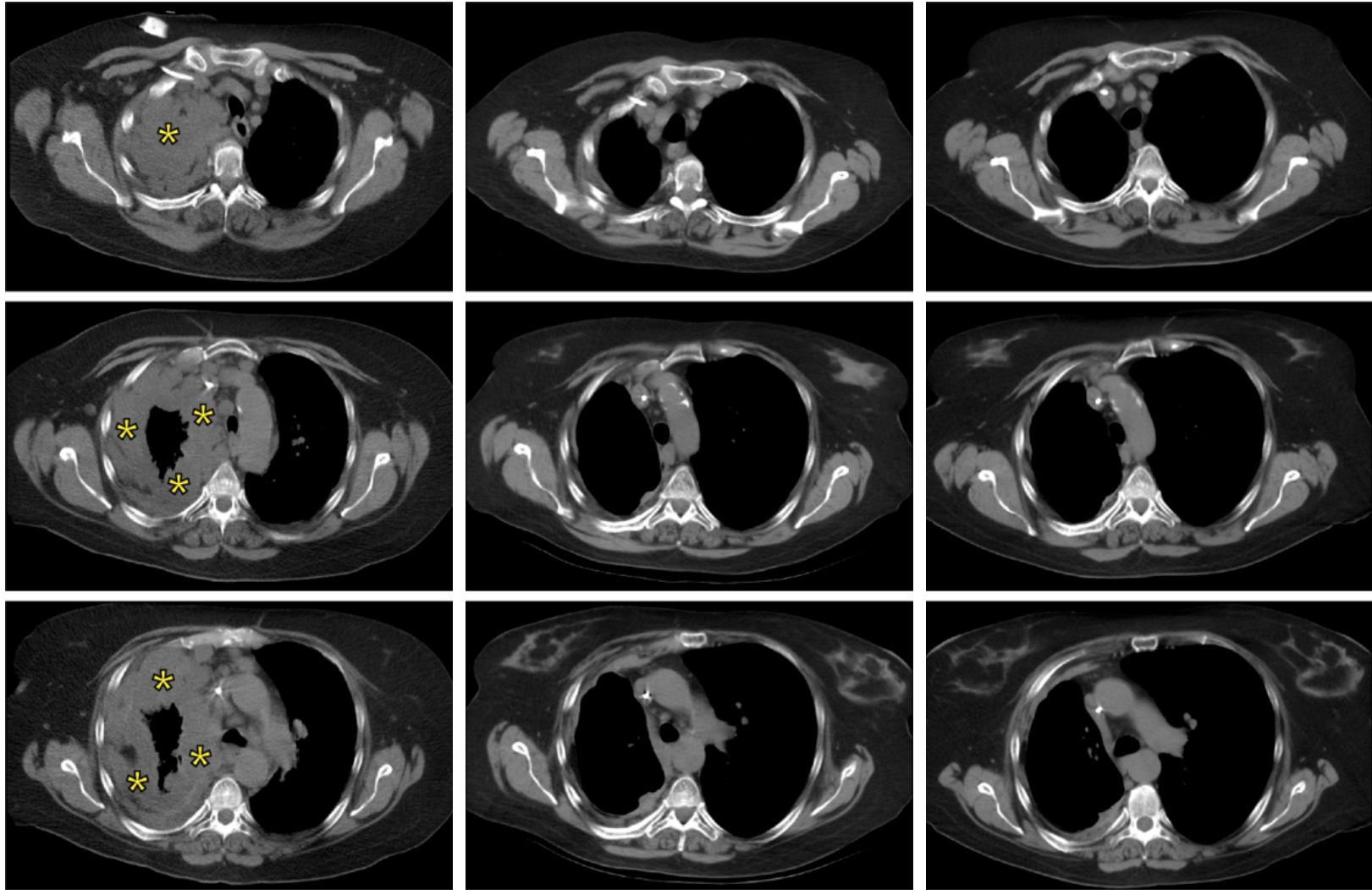
Phase I study of SS1P: 30/34 pts. (88%)

**Present study: 2/10 pts. (20%)
(p=0.0001)**

Tumor responses in patients treated with SS1P plus P/C

Pt.	Tumor response	Delayed tumor response	Post study chemo. received	Response to post study chemo.	Overall survival (Months)
2	Partial response	Yes, at 7 months			35 (alive)
3	Partial response				36 (alive)
4	Stable disease		Yes	Partial response	14
5	Partial response				31
6	Stable disease				8.8
7	Progressive disease				6.2
8	Progressive disease				5.7
9	Progressive disease	Yes, at 4 months	Yes	85% ↓ in FDG uptake	11
10	Progressive disease				4.2
11	Stable disease		Yes	No	7.3

Patient 3 with extensive pleural mesothelioma:



Day 12

3 months

24 months

Patient 5 with widely metastatic peritoneal mesothelioma:

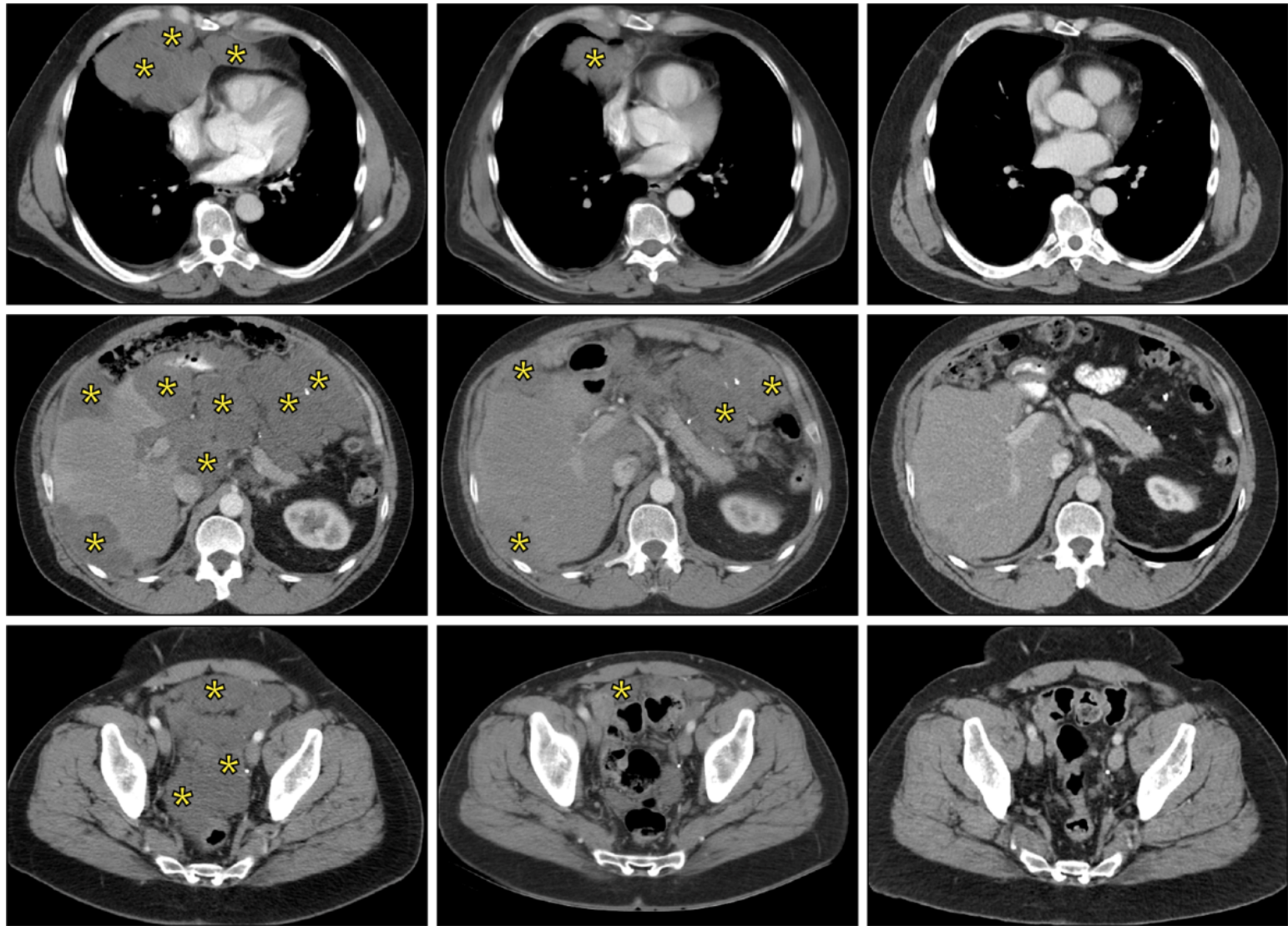


Before treatment



1.6 months

Patient 5 with widely metastatic peritoneal mesothelioma:

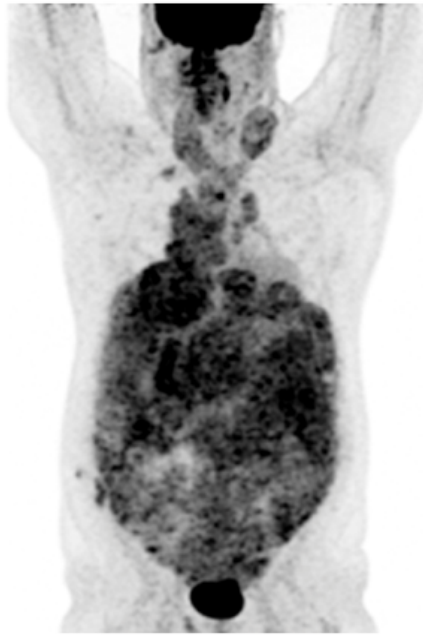


Before treatment

1.6 months

8 months

Patient 5 with widely metastatic peritoneal mesothelioma:



Before treatment

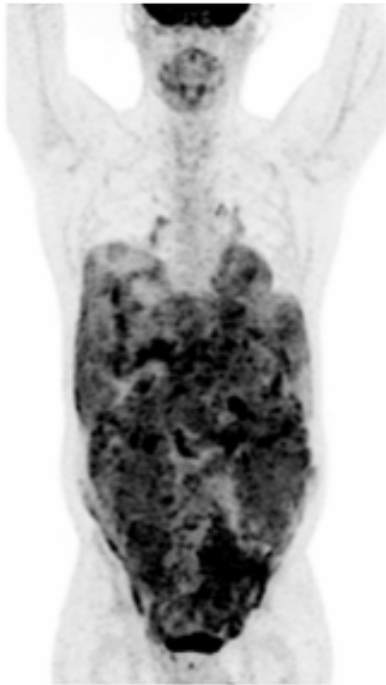


1.6 months



8 months

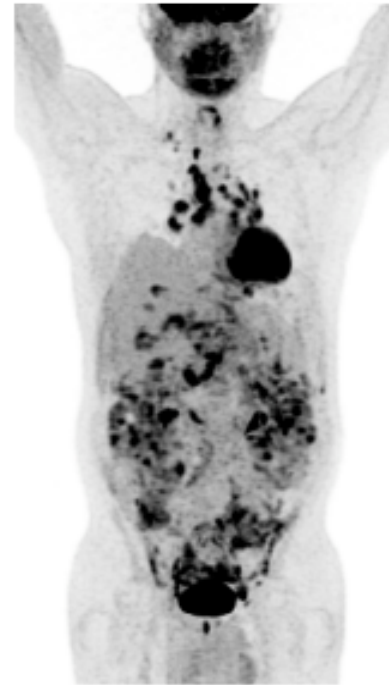
Patient 2 with extensive peritoneal mesothelioma and delayed tumor response:



Before treatment



4 months



14 months

Conclusions

- **SS1P plus pentostatin and cytoxan is safe**
- **Decreases anti-SS1P antibody formation**
- **Dramatic and durable tumor responses in patients with treatment refractory mesothelioma**
- **Doing a small biomarker driven study in mesothelioma to evaluate immune changes within the tumor micro-environment**

Hassan R et al., Science Translational Medicine 2013

PET Response in a patient with extensive mesothelioma (who had received four prior therapies)



August 2014



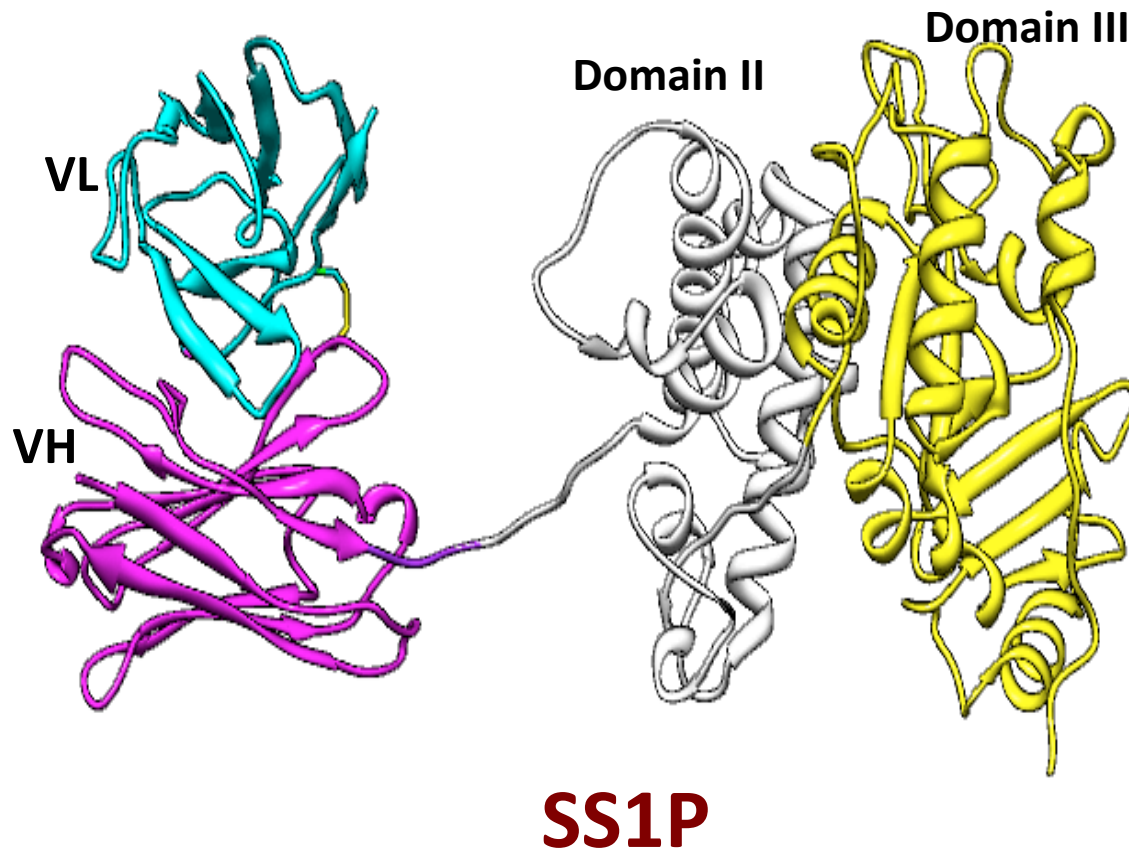
October 2014

However, it would be better to have an immunotoxin that is less immunogenic

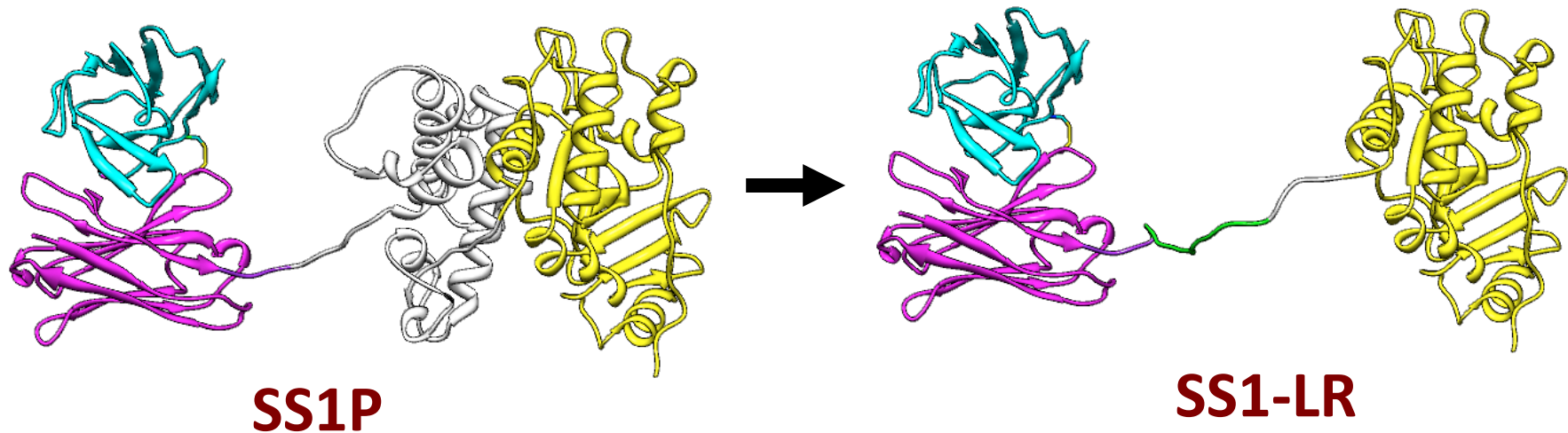
Using protein engineering to make a less immunogenic immunotoxin

Anti-mesothelin Fv

PE38 toxin

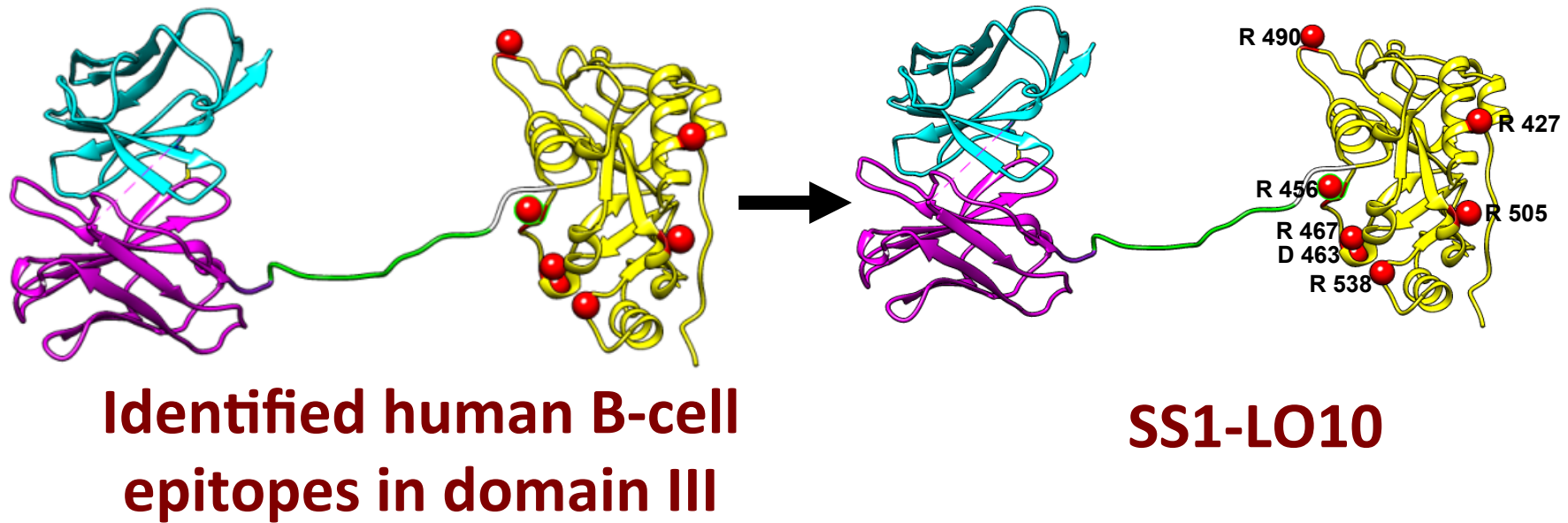


Using protein engineering to make a less immunogenic immunotoxin



- Domain II can be removed without affecting activity
- Removes the main T cell epitopes of PE toxin
- Eight fold less toxic to mice and greatly decreases VLS

Using protein engineering to make a less immunogenic immunotoxin



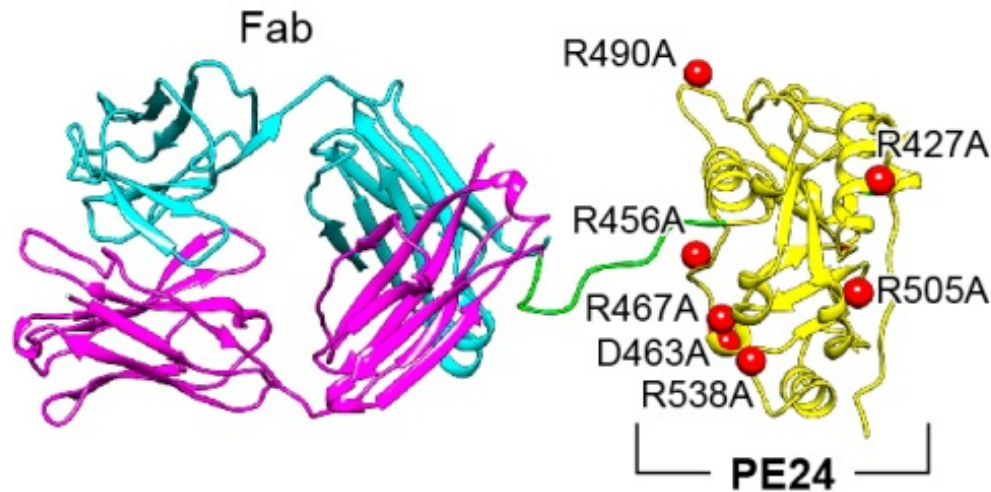
- Removes B cell epitopes and two T cell epitopes of domain III of PE toxin
- Retains cytotoxic activity and less immunogenic

Mazor, Onda, Liu, Pastan

PNAS, 2012
PNAS, 2012

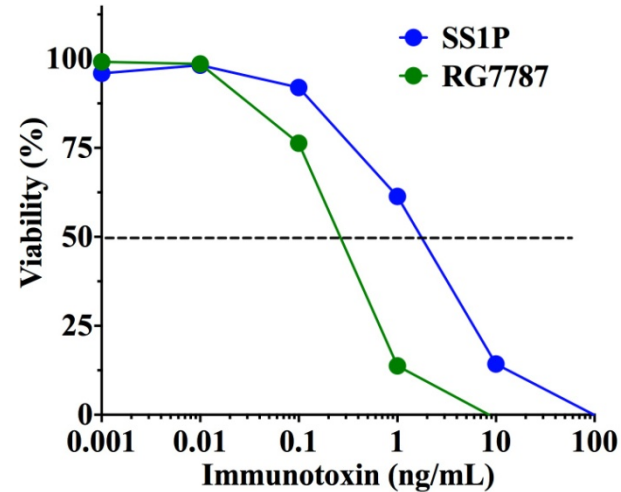
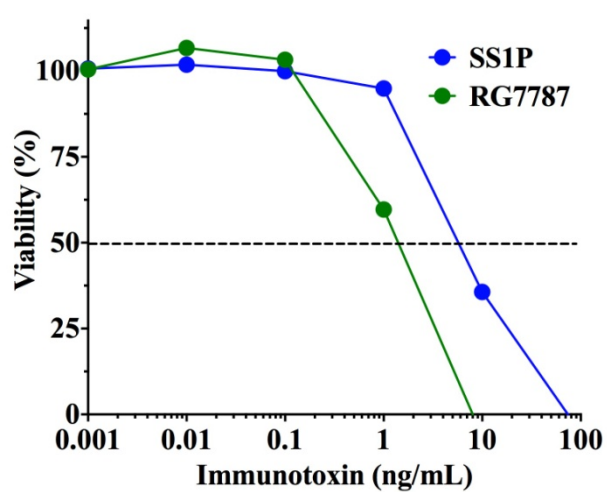
RG7787

A less immunogenic anti-mesothelin immunotoxin



- All known human B cell epitopes and major T cell epitopes of PE have been removed or mutated
- Antigenicity reduced a 1000 fold compared to SS1P
- Can give much higher dose than SS1P and no VLS

RG7787 cytotoxicity against primary mesothelioma cells



Cell lines	Mesothelin Sites /cell (10 ³)	IC50 (ng/ml)	
		SS1P	RG7787
NCI-M-19	41	3.7	1.2
NCI-M-21	56	2.3	0.3

Zhang, Hassan

RG7787

A less immunogenic anti-mesothelin immunotoxin

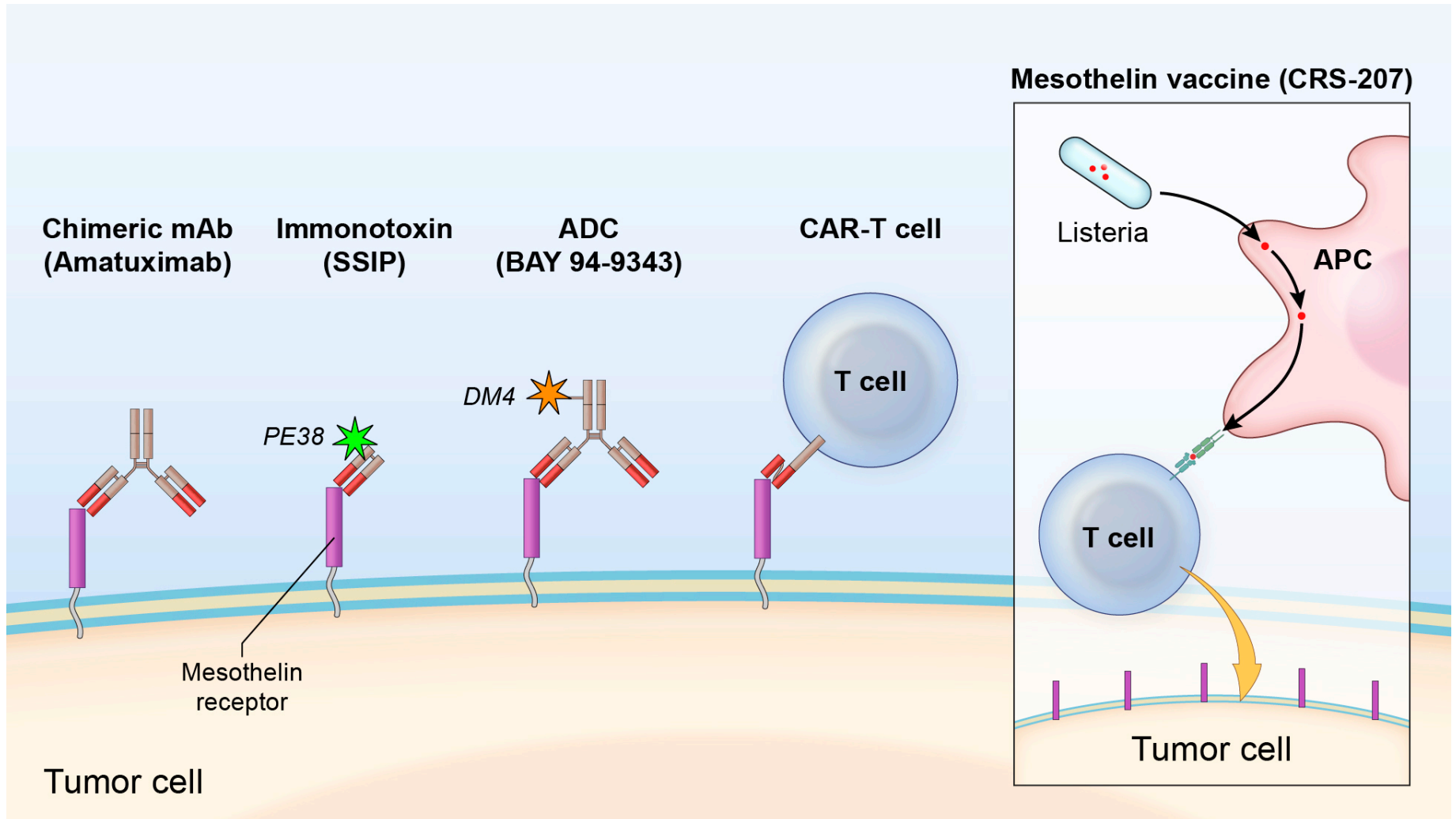
- **Cytotoxic activity against several primary and established cells lines from cancers that highly express mesothelin**
- **Single agent activity against mesothelin positive tumor xenografts in mice**
- **Marked synergy with chemotherapy**

CRADA with Roche for its clinical development

Anti-mesothelin Immunotoxins

- **SS1P with pentostatin and cytoxan has produced significant tumor regressions in treatment refractory mesothelioma patients**
- **Our new generation RG7787 immunotoxin should have more efficacy, because it can be given at higher doses, for more treatment cycles, and have fewer side effects**

Mesothelin directed therapies in clinical trials



Discovery of Mesothelin and Exploiting It as a Target for Immunotherapy



Pastan, Hassan: Cancer Research, 2014

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Bayer Healthcare

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Patients and their families !

